Aquaporins Are Observed in the Duct Epithelia of the Epididymal Region of the Large White Turkey

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ABSTRACT The cellular and molecular mechanisms regulating the reuptake of the testicular fluid supporting sperm exiting the testes in the bird are not known. The presence of aquaporins, proteins involved in transmembrane water transport, was investigated. Observations were limited to the ductuli efferentes, collecting ducts, and ductus epididymis. Interestingly all of these ducts were positive for aquaporins-2, -3, and -9 but not aquaporin-7. When positive, aquaporin was observed localized over the whole cell or the apical plasma membrane of the nonciliated cells and the apical plasma membrane and cilia of the ciliated cells. This study is the first to clearly demonstrate the presence of aquaporins-2, -3, and -9 in the epididymal region of any bird. We assume the aquaporins play a role in concentrating the sperm and in the promotion of sperm maturation in the epididymal region.

(*Key words*: aquaporin, avian, excurrent duct system, sperm maturation, sperm motility)

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INTRODUCTION

The epididymal region is rudimentary in birds and, unlike in mammals, does not have a role in male sperm storage. This region contains the rete testis, ductuli efferentes, connecting ductules, and ductus epididymis. For a detailed discussion of the anatomy and histology of the normal epididymal region of the turkey, refer to Hess et al. (1976). It appears there is some maturation of sperm during transit through the epididymal region, but this is not crucial for sperm to penetrate and fertilize an ovum (Froman, 1994). More likely, the absorption of testicular fluid by the epididymal region and the resulting increase in sperm concentration is the primary function of this collection of small ducts. The cellular and molecular mechanisms governing this fluid transfer have yet to be elucidated.

The movement of water across cell membranes is accomplished by simple diffusion through the lipid bilayer and by bulk flow driven by an osmotic gradient through hydrophilic pores or channels. The transmembrane water channel proteins responsible for the water flow were identified more than a decade ago and are referred to as aquaporins (AQP) (Preston and Agre, 1991). Ten AQP homologues have been cloned so far in mammals, and other AQP have been identified in amphibians, plants, yeast, bacteria, and various lower organisms (Verkman and Mitra, 2000). They are widely distributed and more than one AQP could be present in the same cell. All AQP seem to have 6 transmembrane domains comprised of 5 connecting loops with the amino and carboxyl terminals in the cytoplasm. They are synthesized as monomers (monomer size ~30 kDa), but there is evidence suggesting that AQP are formed in the membrane as tetramic units, each of which has 4 water pores (Echevarria and Ilundain, 1998). The AQP are members of the major intrinsic protein superfamily of integral membrane proteins and have been divided into 2 subgroups according to their transporting characteristics. The first group, the *aquaporins*, is water selective and consist of AQP-0, -1, -2, -4, -5, and -6. The second subgroup, *aquaglyceroporins*, is permeable to water and to small molecules such as urea and glycerol and consist of AQP-3, -7, and -9.

The transport properties of some of the AQP appear to be species dependent. Urea conductance of AQP-8 has been identified in mouse (Ma et al., 1997) but not in rat (Koyama et al., 1997). Ma et al. (1997) also reported that mouse AQP-8 conducts water and urea but not glycerol. In humans, AQP-8 has been found to be a water selective channel without permeability to urea or glycerol (Koyama et al., 1998). Similar species differences in transport properties have also been encountered in other AQP, e.g., AQP-9 (Elkjaer et al., 2000). Recent studies have suggested

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Abbreviation Key: AQP = aquaporin.

that AQP may also be permeated by gases (Cooper and Boron, 1998). In addition, expression of AQP-9, a likely candidate for apical transepithelial fluid and solute transport in several regions of the male reproductive tract, is modulated by androgens in the adult rat epididymis (Pastor-Soler et al., 2002).

It is not known if AQP play a role in fluid absorption in the avian epididymal region. In the following study, we used immunocytochemistry to determine the presence or absence of various AQP in the epididymal region of the turkey.

MATERIALS AND METHODS

Experimental Birds and Tissue Fixation

Large White turkey males[2] 28 to 55 wk of age were maintained under standard husbandry conditions with feed and water provided ad libitum. Males were photostimulated at 26 wk (14L:10D) and were in semen production by 29 wk of age. Six toms in semen production for 2 to 6 wk were euthanized with an intravenous dose of sodium pentobarbital,[3] and the testes and epididymal region from the left testicle were isolated. Excised tissue samples about 2 to 3 mm^3 were fixed in 4% paraformaldehyde[4] in PBS at 5°C. After 24 h, the fixed tissues were transferred to PBS and stored at 5°C until further processing.

Immunoperoxidase Methods

Fixed tissues were embedded in paraffin, and 5 μm thick sections were mounted on Fisher Superfrost Plus[5] slides. Sections were deparaffinized in xylene and rehydrated in decreasing grades of ethanol solution (95, 70, and 50%). The endogenous peroxidase activity was blocked by immersing the slides in 1.5% H_2O_2 in ethanol for 30 min at room temperature. The slides were rinsed with PBS and blocked overnight at 5°C with 1% milk.

The antirabbit-AQP antibodies were obtained from Alpha Diagnostic International.[6] All antibodies were diluted 1:200 in PBS containing 0.4% polyvinyl pyrolidone[4] with 1% bovine serum albumin as stabilizer, and all the sections were incubated for 12 h at 5°C. After being rinsed with PBS, the sections were incubated with Biotinylated Universal Secondary Antibody[7] for 2 h at room temperature and subsequently with avidin-biotinylated-peroxidase complex[7] for 30 min at room temperature. The immunohistochemical reaction was visualized by using Vector VIP[7] and H_2O_2 as substrates. The sections were incubated until suitable staining developed (generally 15 min). After being rinsed with tap water for 5 min, sections were dehydrated in ethanol (95% and 100%) for 3 min each followed by 3 changes of xylene for 3 min each. The sections were then mounted and examined using a Zeiss Axioskop Microscope.[8] Specificity of the immunostaining was confirmed by following the above procedures in the absence of the primary antibody (negative controls). The sections were digitally imaged using a MC80 Zeiss Microscope Cameras[8] and Bioquant BQ-TCW98 software. Final images were imported into MGI Phosuite III SE.

[2]British United Turkeys of America (BUTA), Lewiston, WV.
[3]A. J. Buck & Son, Cockeysville, MD.
[4]Sigma, St. Louis, MO.
[5]Fisher Scientific, Pittsburgh, PA.
[6]San Antonio International, San Antonio, TX.
[7]Vector Laboratories, Burlingame, CA.
[8]Carl Zeiss Inc. Microscope Division, Thornwood, NY.

TABLE 1. Expression of aquaporins (AQP) in the epididymal region of the mature turkey[1]

Region	AQP-2	AQP-3	AQP-7	AQP-9
Ductuli efferentes	+, ++	++	–	+, ++
Connecting ductules	+	++	–	+, ++
Ductus epididymidis	+	+	–	++

[1]Number of plus (+) signs is directly proportional to the intensity of the reaction, with (+) light reaction, (++) intense reaction, and (–) absence of reaction.

RESULTS AND DISCUSSION

Our observations were limited to the ductuli efferentes, connecting ductules, and ductus epididymis. All of these

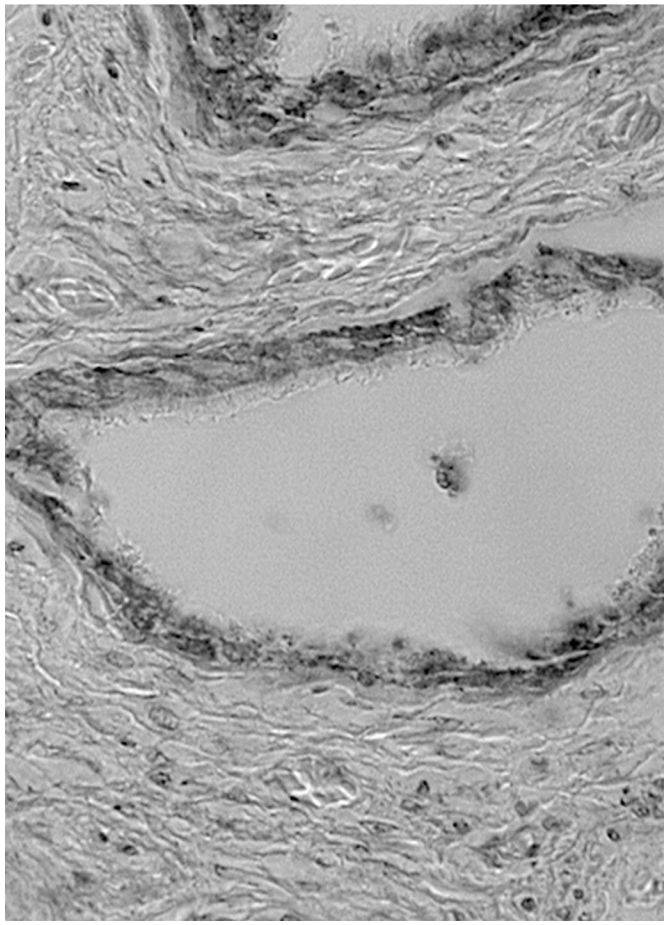

FIGURE 1. Ductule efferentes epithelial cells are lightly positive for aquaporin-2. Magnification = 630×.

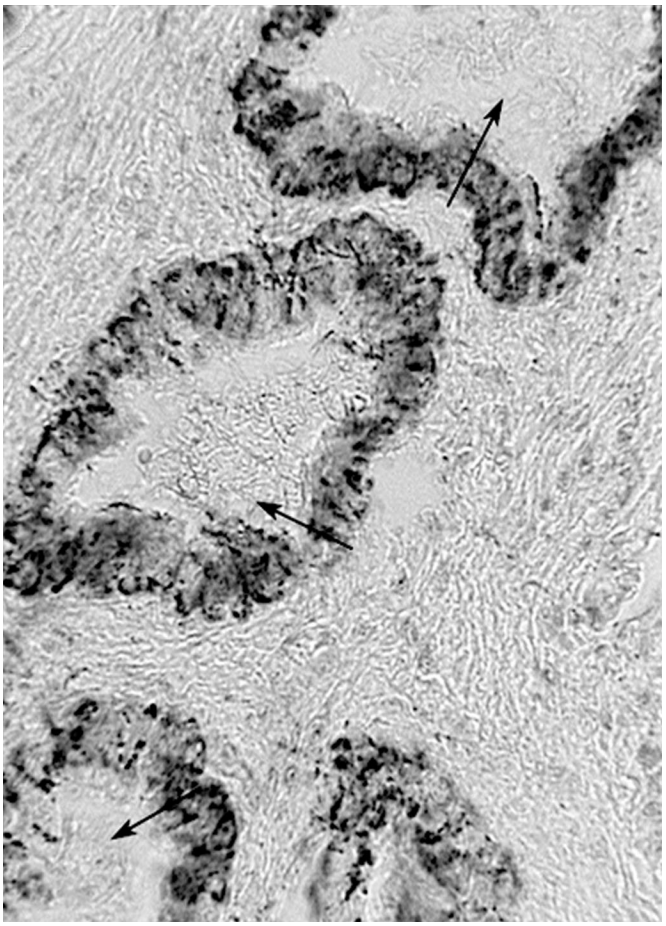

FIGURE 2. Connecting ductule epithelial cells, particularly their basal-lateral faces, are strongly positive for aquaporin 3. Arrows show luminal sperm. Magnification = 630×

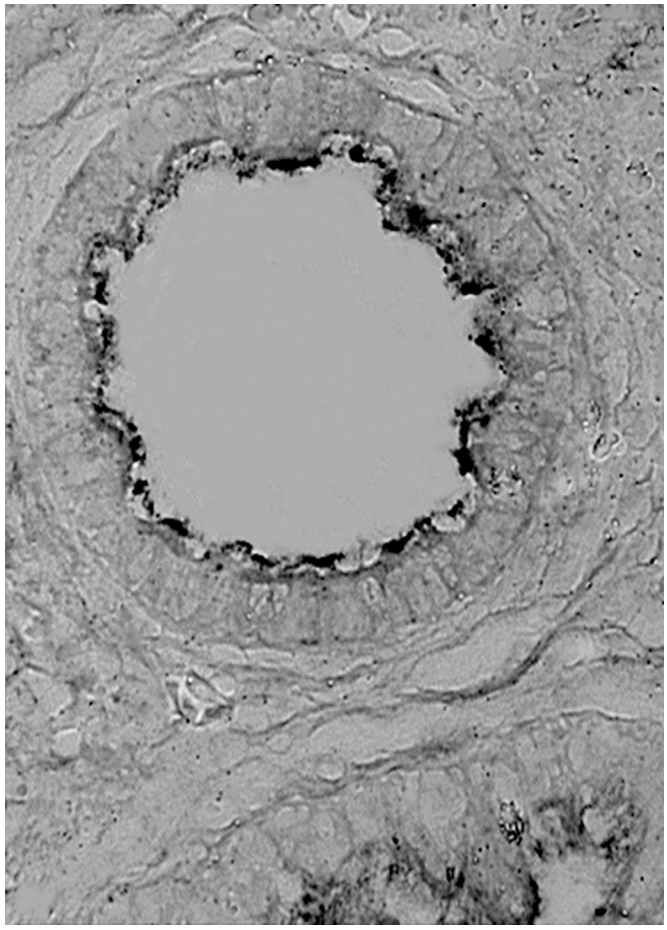

FIGURE 3. The apical surface of the ductule efferentes epithelial cells stain strongly with antiaquaporin- 9 antibody. Magnification = 630×.

ducts were positive for AQP-2, -3, and -9 and negative for AQP-7 (Table 1). When positive, reaction product covered the entire epithelial cell or was observed to be localized to the apical plasma membrane of the nonciliated cells and the apical plasma membrane and cilia of the ciliated cells (Figures 1 to 3). Although all negative controls failed to exhibit any reaction product (Figure 4), faint positive staining of the loose connective tissue surrounding the ducts in the epididymal region was a consistent feature in all sections positive for AQP.

The ducts of the epididymal region function, in part, to resorb testicular fluid and subsequently concentrate the sperm prior to transport to the ductus deferens (Froman, 1994). This appears to be coupled with the sperm acquisition of secretory proteins and the onset of more vigorous sperm motility during passage through the epididymal region (Esponda and Bedford, 1985). The localization of AQP -2, -3, and -9 coupled with the fenestrated endothelium of the capillaries (Nakai et al., 1988) surrounding the ductuli efferentes, collecting ducts, and ductus epididymidis further support the concept of a vigorous fluid exchange within this region. These fluid exchange mechanisms may account for the 60-fold increase in sperm concentration observed between the seminiferous tubules and the ductus deferens (Clulow and Jones, 1982). Froman (1994) suggested that 92% of the luminal fluid absorption in Japanese quail is accomplished within the ductuli efferentes.

Aquaporin-2 is the vasopressin-regulated water channel expressed exclusively in collecting ducts of the kidney (Agre et al., 1995; Fushimi and Marumo, 1995). This protein is also found in the testis and epithelial lining of the vas deferens of transgenic mice (Nelson et al., 1998). AQP-3 was first detected at the basolateral membranes of collecting duct cells of the rat kidney (Echevarria et al., 1994; Ishibashi et al., 1997). AQP-9 mRNA was first detected in hepatocytes, immature spermatocytes, and Leydig cells in rat testis (Tsukaguchi et al., 1998) and in the rat epididymis (Elkjaer et al., 2000; Pastor-Soler et al., 2001; Badran and Hermo, 2002). AQP-9 in different segments of the male reproductive tract may assist in maintaining water equilibrium within these cells. AQP-9 also allows the passage of solutes such as polyols, purines, and pyrimidines (Tsukaguchi et al., 1999).

In summary, our data show that AQP-2, AQP-3, and AQP-9 are present in the turkey epididymal region. These AQP could represent an important pathway for transepithelial water movement out of the duct lumina, thereby increasing sperm concentration. Likewise, AQP may also contribute to the transfer of specific solutes into the duct

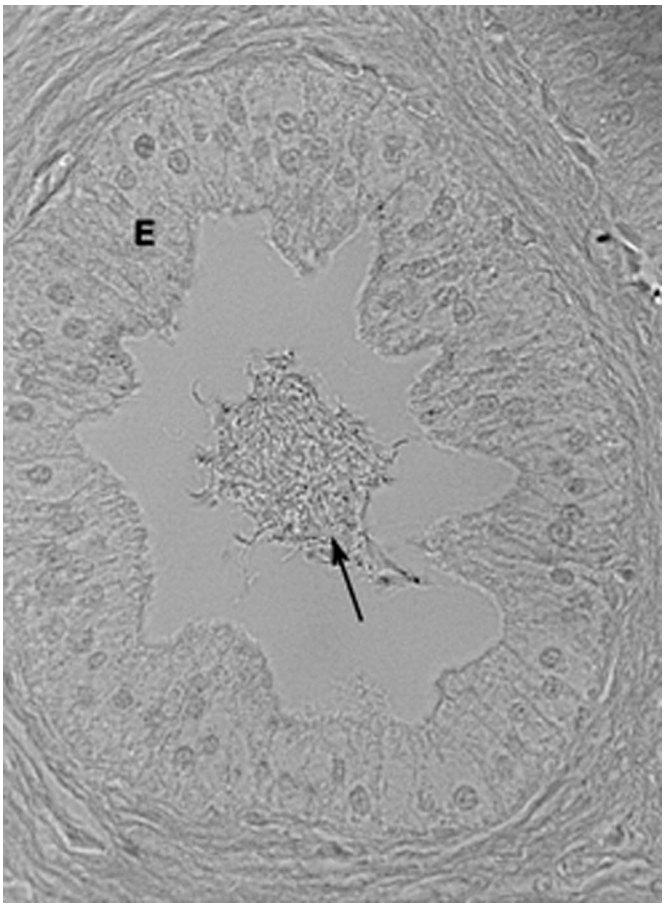

FIGURE 4. No staining was observed in the absence of the primary antibody. Arrow shows sperm in the lumen of a connecting ductule lined by columnar epithelial cells (E). Magnification = 630×.

lumina associated with sperm acquisition of motility and maturation (Tsukaguchi et al., 1998).

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